Appreciation survey of natural biodegradation of crude oil which cause soil contamination and trying to diagnose the bacterial species which cause this biodegradation

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Abstract

This survey includes proven analysis of polluted soils of Kirkuk crude oil for three sites in Baiji refineries trying to know the bacterial species which are responsible on biodegradation and it's quantity on the crude oil.

Samples of polluted soils were collected with the control samples for four seasons during 2002 and 2003 and they were analyzed. The survey indicates the following:

1-Adecreasing of pollution ratio was happened by the time where the ratio of the biodegradation during the four seasons starting from winter 2002 to Autumn 2003 was 18.5 %

2-Adifference among the isolated bacterial species and that was according to the temperature degrees which were used in incubation of soil samples. *Acinetobacter SPP* was the predominant during the four degrees of incubation.

3-*Flavimonis oryzihabitans* makes the biggest biodegradation when the sample was incubating on 20C° where the ratio of decomposition was 68.269%, also the same kind makes the biggest decomposition ratio during incubation in 25C° where the ratio was 56.724% comparing with *Acinetobacter SPP* .

While incubation in 30C°, just *Acinetobacter SPP*. was decomposed where its ratio was 51.335%. On the other hand, during incubation in 35C° *Aeclerica SPP* makes the biggest decomposition ratio which was 72.296% compared with the other two kinds

introduction

In Iraq, oil fields which have a wide distributions in many sites linked together by a wide net of transfer pipes to carry the oils to all refiners and exporting ports in north and south of Iraq but the accidents destroy these pipes so, a large quantity of oils run over the soil and cause soil pollution, therefore we choose Baiji refinery which situated in the north of Iraq because it is the largest and causing a large contamination in the soil , Baiji refinery is receiving the crude oil from Kirkuk fields which situated in the east of Iraq, during the last four years after American inroad in Iraq the pipe line which carrying the crude oil from Kirkuk fields to Baiji refinery was exposed to many terrorism processes and a large quantity from crude oil was flowed and many thousand acre were polluted. The crude oil is playing important role in the life and when the human start to use un reborn materials such as the crude oil, the environmental pollution problems were started and this problem become a proper dander threaten human life (1 ,2 and 3).

Pollution problems by crude oil are increasing dramatically during the last century because of the increasing of production and consumption processes especially at the last thirty years, the international production from the crude oil at (1979) was(3084 million metric ton) and only Iraqi production at this year was (175 million metric ton)(4) While the international production at (2005) was (26645 million metric ton) (5)

Only Iraqi production at this year was (730million barrel) (6). (7) It is said that, in the world there are about (14000) flow cases happened yearly to biosphere and this explain the danger which resulted from the arrival of very large quantity of crude oil to biosphere. The frequently accidents affects the environmental characteristics and the organisms' life which living at contaminated sites with oily compounds especially (Benzene, Toluene, Ethylbenzen, Xylene) which have a very poisoning properties (8) In general the effects of crude oil components have wide effects on the environment and human health (9) On the other hand the contaminated soil with crude oil will become unsuitable for the human Inhabiting and agriculture also the pollution of soil may leads to water pollution which is a dangerous problem threatening human health especially the countries that using ground water as a drinking water (10)

Many main exchanges will be happen to the chemical and physical properties of the soil which is contaminated by crude oil and these exchanges is depended on the biological activities inside the soil which include the microorganisms and plant roots . The high concentrations of non saturated and aromatic compounds that make the crude oil are more poisoning because they penetrate inside the plant tissues and also affects on seeds germination (11).

crude oil composition

The crude oil is a complex mixture of many thousand hydrocarbon compounds linked together. These compounds are classified into saturated and aromatic compounds (12, 13) .The aliphatic compounds are the main component for many crude oil types and more dispersing at nature (14) these compounds are more exposed to decomposition by the action of bacteria and fungus and there are another compounds which more stable in the environment such as the resins and asphalt (15).

The hydrocarbons divided to three groups by depending upon the carbon Atom number.

A- Light Hydrocarbons $C_1 - C_{10}$

B- Medium Hydrocarbons $C_{11} - C_{35}$

C- Heavy Hydrocarbon C_{36}- C_{50}

According to the hydrocarbon molecules arrangement the aliphatic hydrocarbons is arranging in straightforward compounds while the aromatic hydrocarbons is arranging in hexagonal cycles. The aromatic hydrocarbons is divided into monoaromatic which contains a one cycle such as (Benzene, Toluene, Ethylbenzen and Xylene), diaromatic contains two cycles while multiaromatic contains more than two cycles (16).

All the facts indicate that production and using of oil are precipitated in the oil contamination (17) On the other hand (18).It is pointed that in LOWA which is an

American state there are more than (3500) sites oil leaked from, (19) also it is said that the soil contamination problems by crude oil are coupling with the presence of the refineries diffusion ,there are another reasons which help the oily pollutant to penetrate the soil layers such as the road accidents , leaks from the pipes and oil storages and during the oil fields fire(20).

Biodegradation of crude oil

The large part of organic materials is degraded by the action of bacteria and converted to other forms which become more stable and not polluted (21, 22). The microorganisms is playing important role in analyzing the organic materials in the soils and another sediments to inorganic materials such as

Carbon, Nitrogen, Phosphorous, sulfurs, and other elements, this process is called (Mineralization) (23). The biodegradation of crude oil hydrocarbons depends on the direct contact between the hydrocarbons and the surface of the bacteria (24). The crude oil molecules have a limited move and attach strongly at the soils surface therefore we must use the biological treatment to remove these pollutants because the crude oil hydrocarbons is staying at the upper six foots of the soil surface near the plants root (25 , 26). The soil microorganisms growth is affected by many factors such as nutrition minerals, growth factors, ionic content, water, temperature, pressure, radiation, all these factors have a maximum, minimum and optimum circumstances (27) There are other factors which play important role in increasing the activation of the bacteria and degradation of crude oil such as the mixing of contaminated and uncontaminated soil. Adding of fertilizers to stimulate the bacteria to degrade the crude oil (28) ,the remaining and persistent of the organic pollutants in the soil and ground water depend on many factors to help the microorganisms to execute there actions(29).

1- The presence of the bacteria which can live in side the contaminated area

2- The ability of the bacteria to degrade the hydrocarbons

3- The ability range of the hydrocarbons to degrade

4-the availability rang of nutrition which is necessary for bacterial activities

5- The environmental factors around the contaminated area

The attacking of the bacteria of oil hydrocarbons gives the concept

Of biological treatment for oil pollution (30, 31), found that the

Acinetobacter venetianus will adherenton diesel fuel molecules and forming a chemical compound from polysaccharides which consists up of glucose and mannose. the study of micro composition of the polysaccharides situation in the surface of bacteria in presence of diesel fuel showed occurring two types from (Lectins) are (PNA and Con A) , diesel fuel(C_{J2} - C_{28}) which contain a mixture of (n - alkanes) is consuming of the following bacteria species (*Acinetobacter venetianus ,Pseudomonas putida ,Alcaligenes feacalis)* which is used as a main source for carbon , energy and oxidation of(n - alkanes) to (n-alkanose and n- alkanoates) (32), the bacteria species are tracking many rotes to degrade the (Toleune) and swimming to the pollutants through it's sensitivity by the presence of the poisonous compounds such as (Toleune , Benzen and Trichloroethylene) and move by chemo taxis to the contaminated site(33).

On the other hand the presence of the elements in the soil at some concentration will considered a positive indicator for the microorganisms activation in the soil and when these concentration become more than the natural values that mean the soil is contaminated and have a negative effect on the soil microbial equilibrating and on the effective of these organisms , (40%) from contaminated sites with dangerous residues at united states of America are contaminated by organic pollutants and metallic pollutants, there are many available methods to estimation the quantity of the crude oil hydrocarbons which degradation by the action of microorganisms such as the total viable count and most probable number for the microorganisms which act to degradation the different hydrocarbon compounds (34 , 35) ,also there are chemical methods such as different chromatography techniques (36 ,37)and by using infra red rays (38).

Materials and methods

The three Sites which were exposed to oil contamination in Baiji refinery were limited, three samples of the soil were collected from each site during four seasons at 2002-2003 in plastic sacks, and the oil contamination was determined for each sample and counts the average of the biodegradation

at each season , unpolluted samples from soil were brought from the same refinery to isolate the germs which leads to decomposition of the crude oil hydrocarbons and diagnose by using (BioMerieux Api 20*) which supplied from (Iprime EN company) the results were recorded .

media preparation

Mineral Salt medium :-this medium prepped at laboratory by the method of (39) by dissolving (0.5 grm NI^Cl) , (4 grm NaCl) (O.Sgrm KH_2PO_4) (0.5 grm $MgSO_4$) in (One litter of Distilled water and the PH were mended to (7.2) , the media sterilized by Autoclave at (121 C°) for (15 minutes) the crude oil was added at ratio (1% and 2%) as a main source for carbon and energy.

Bacteria digenesis

The bacteria were digenesis at bacteriological department at Al-Kinndi hospital , the species were isolated by using two media (blood agar & MacConkey agar) by method (BioMerieuxkits) , different species of isolated bacteria were grown at tubes which content (T.S.B) and incubated at 37C° for 24 hours.The isolated bacteria were grown on crude oil as a main source of carbon and energy

Results

Three contaminated sites with crude oil were delimitated at Baiji refinery which receiving the crude oil from Kirkuk fields, the ratio of the contamination was measured, and the measuring is recording seasonally after saving the sites from recontamination, the results at table (1) showed that the contamination ratio is decreasing with the time and the average of biodegradation ratio during one year (18.50 %)

Table: (1) the natural field contamination and the seasonal biodegradation which happened in contaminated soil samples

No	Sample No	Contamination ratio%	The time				Biodegradation ratio %
			winter	spring	Summer	Outman	
1	Bl	0.52	0.51	0.48	0.45	0.43	17.31
2	B2	0.36	0.36	0.33	0.30	0.29	19.45
3	B3	0.48	0.47	0.44	0.42	0.39	18.75
		The average biodegradation ratio%					18.50

Isolation and digenesis the bacteria which decompose the crude oil

After the soil fertilization by the crude oil which product from Kurkuk fields incubated at 20C° , for 21 day the most species which grow were:(*Flavimonas oryzihabitans , Acinetobacter spp, Stenotrophomonasmaltophilia)* while when the soil incubated at 25C° for the same time the most species which grow were(*Acinetobacter spp* .and *Flavimonas oryzihabitans}.*

On the other hand when the same sample incubated at 30C° for 21days only *Acinatobacter spp* was grew but when the same samples incubated at 35C° for 21 days the three species grow

(*Acinetobacter spp , Pasteurella spp* and *Leclerica spp*) as in tables (2,3,4 and 5) .

Table (2) the percentage ratio for the decomposition of Kirkuk crude oil by the action of three species of bacteria which were incubated at 20C°

No	The bacteria which treated with crude oil	Weight ($1cm^3$) untreated crude oil (grm)	Residue weight with out analysis (grm)	Analysis weight by the action of bacteria(grm)	Percentage ratio for for analysis of crude oil
1	*Acinetobacter spp*	0.8462	0.4560	0.3902	46.112
2	*Stenotrophomonas maltophilia*	0.8462	0.3544	0.4918	58.118
3	*Flavimonas oryzihabitans*	0.8462	0.2685	0.5777	68.269

Table (3) the percentage ratio for the decomposition of Kirkuk crude oil by the action of two species of bacteria which were incubated at 25C°

No	The bacteria which treated with crude oil	Weight ($1cm^3$) from untreated crude oil(grm)	Residue weight without analysis(grm)	Analysis weight by the action of bacteria(grm)	Percentage ratio for analysis of crude oil
1	*Acinetobacter spp*	0.8462	0.4323	0.4139	48.912
2	*Flavimonas oryzihabitans*	0.8462	0.3662	0.4800	56.724

Table (4) the percentage ratio for the decomposition of Kirkuk crude oil by the action of one species of bacteria which were incubated at 30C°

No	The bacteria which treated with crude oil	Weight ($1cm^3$ from untreated crude oil(grm)	Analysis weight by the action of bacteria(grm)	Analysis weight by the action of bacteria(grm)	Percentage ratio for analysis of crude oil
	Acinetobacter spp	0.8462	0.4118	0.4344	51.335

Table (5) the percentage ratio for the decomposition of Kirkuk crude oil by the action of three species of bacteria which were incubated at 35 C°

No	The bacteria which treated with crude oil	Weight (Icm3) untreated crude oil(grm)	Residue weight without analysis (grm)	Analysis weight by the action of bacteria (grm)	Percentage ratio for analysis of crude oil
1	Acinetobacter spp	0.8462	0.2126	0.6339	74.875
2	Pasteurella spp	0.8462	0.3521	0.4941	58.390
3	Leclerica spp	0.8462	0.2344	0.6118	72.299

a viable account for bacteria which analyze the crude oil

The a viable account of bacteria which was isolated from local soil and grow on mineral salt medium and nutrient agar by using many incubation temperature degree :-

incubation at 20,25,30 and 35C°:- the a viable account for the bacterial colonies were done at (20,25,30 and 35) for (28 day) and the results at the tables (6,7,8 and 9) are showing the growth of the bacteria which is responsible for the decomposition of crude oil which is produced from Kirkuk fields .The growth of the species is increasing with the incubation time.

Table (6) a viable account (cell/ml) for the bacteria which is responsible for decomposition of Kirkuk crude oil and that is incubated at 20C° for 28 day

No	Growing bacteria	The time				
		Zero time	After one weeks	After two weeks	After three weeks	After four weeks
1	Acinetobacter spp	18	424	61	3605	3812
2	*Stenotrophomona s maltophilia*	41	652	416	1484	1566
	Flavimonas oryzihabitan	36	128	312	3125	5682

Table (7) a viable account (cell/ml) for the bacteria which are responsible for decomposition of Kirkuk crude oil and that is incubated at 25C°for 28 day.

No	Growing bacteria	The time				
		Zero time	After one weeks	After two weeks	After three weeks	After four weeks
1	*Acinetobacter spp*	12	248	286	1560	2432
2	*Flavimonous oryzihzbitan*	22	356	351	1452	2106

Table (8) a viable account (cell/ml) for the bacteria which are responsible for decomposition of Kirkuk crude oil and that is incubated at 30C° for 28 day .

No	Growing bacteria	The time				
		Zero time	After one weeks	After two weeks	After three weeks	After four weeks
1	*Acinetobacter spp*	34	166	259	1323	2892

Table (9) a viable account (cell/ml) for the bacteria which are responsible for decomposition of kurkuk crude oil and incubated at 35 C^{o} for 28 day.

No	Growing bacteria	The time				
		Zero time	After one weeks	After two weeks	After three weeks	After four weeks
1	Acinetobacter spp	٤	٥٦	٢١٤	١٤٦٠	٣٢١٤
٢	Pasteurella spp	٢٨	٨٢	٣٥٢	١٧٨١	٢٨٦٦
٣	Leclerica spp	٩	٧٤	٢٨٨	١٥١٤	٣٠٤١

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دراسة تقدير التحلل الحيوي الطبيعي للنفط الخام الملوث للتربة و محاولة تشخيص الانواع البكتيرية التي تساهم بذلك

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الملخص:
تضمنت الدراسة إجراء تحليلات مختبريه على ترب ملوثة بنفط كركوك الخام لثلاث مواقع في مصافي مدينة بيجي لمحاولة معرفة الأنواع البكتيرية المسؤولية عن التحلل الحيوي و كمية التحلل الحيوي على النفط الخام.جمعت عينات الترب الملوثة وكذلك تربة المقارنة للفصول الأربعة خلال عامي ٢٠٠٢و٢٠٠٣ وأجريت عليها التحليلات المخبريه وأظهرت الدراسة ما يلي
١-حصول انخفاض في نسبة التلوث مع الزمن إذ بلغ معدل التحلل الحيوي خلال الفصول الأربعة ابتداء من فصل الشتاء لعام ٢٠٠٢ لغاية خريف ٢٠٠٣ ما مقداره ١٨,٥ % .
٢-حصول اختلاف في الأنواع البكتيرية المعزولة وذلك تبعا لدرجات الحرارة المستخدمة في حضانة عينات التربة وكان النوع *Acinetobacter* SPP هو السائد خلال درجات الحضانة الأربعة.
٣-احدث النوع *Flavimonis oryzihabitans* اكبر تحلل حيوي عند حضن العينة لدرجة حرارة ٢٠ مئوية إذ بلغت نسبة التحلل ٦٨,٢٦٩% كما سبب النوع نفسه اكبر نسبة تحلل عند حضن العينة لدرجة ٢٥ مئوية إذ بلغت نسبة التحلل ٥٦,٧٢٤% مقارنة بالنوع Acinetobacter *SPP* و عند حضن العينة بدرجة حرارة ٣٠ مئوية فقد انفرد النوع *Acinetobacter Spp* بالتحلل إذ بلغت نسبة التحلل ٥١,٣٣٥ % أما عند الحضن بدرجة حرارة ٣٥ مئوية فقد سبب النوع *Aeclerica SPP* اكبر نسبة تحلل بلغت ٧٢,٢٩٦ % مقارنة بالنوعين الآخرين.